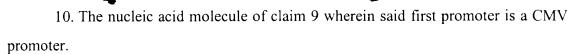


10

15

20

- 1. An isolated nucleic acid molecule comprising
- (a) a first class switch region (S_1) nucleotide sequence of an upstream immunoglobulin locus under transcriptional control of a first promoter;
- (b) a second class switch region (S₂) nucleotide sequence of an immunoglobulin locus downstream of said upstream Ig locus under transcriptional control of a second promoter, wherein said S₂ sequence serves as a region-specific substrate for class switch recombination (CSR);
- (c) a reporter gene nucleotide sequence encoding a reporter molecule, interposed between said S_1 and S_2 sequences in reverse transcriptional orientation, and
- (d) a promoter, downstream of said nucleotide sequence encoding said reporter molecule, allowing the expression of said reporter molecule only following CSR between said S₁ and S₂ sequences.
- 2. The nucleic acid molecule of claim 1 wherein said S_1 is an $S\mu$ sequence and said S_2 is an $S\gamma 2$ sequence.
- 3. The nucleic acid molecule of claim 1 wherein said S_1 is an $S\mu$ sequence and said S_2 is an $S\epsilon$ sequence.
- 4. The nucleic acid molecule of claim 2 wherein said S_1 and S_2 sequences are G-rich switch region DNA sequences.
- 5. The nucleic acid molecule of claim 3 wherein said S_1 and S_2 sequences are G-rich switch region DNA sequences.
- 6. The nucleic acid molecule of claim 1 wherein said nucleic acid in part (c) and said promoter in part (d) are under control of an internal ribosome entry site (IRES).
- 7. The nucleic acid molecule of claim 1 wherein said nucleic acid in part (c) encodes a Green Fluorescent Protein (GFP) molecule.
- 8. The nucleic acid molecule of claim 1 wherein said nucleic acid in part (c) encodes a reporter molecule selected from the group consisting of β -galactosidase, luciferase, and secreted alkaline phosphatase (SEAP).
- 9. The nucleic acid molecule of claim 1 wherein said first and second promoters are non-inducible constitutive promoters.



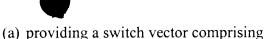
- 11. The nucleic acid molecule of claim 9 wherein said second promoter is an SV promoter.
 - 12. An isolated nucleic acid molecule comprising

10

15

20.

- (a) a human Sµ nucleotide sequence under control of a CMV promoter;
- (b) a human S_{γ_2} nucleotide sequence under control of an SV promoter;
- (c) an RSV LTR enhancer/promoter and GFP gene under control of an internal ribosome entry site (IRES), interposed between said $S\mu$ and $S\gamma_2$ sequences, in reverse transcriptional orientation,
- (d) a 5' splicing donor site from human β -globulin gene, 3' of said $S\mu$ sequence; and
- (e) a 3' splicing acceptor site and C ϵ 1 exon, 3' of said S γ_2 sequence.
- 13. The nucleic acid molecule of claim 12 further comprising a nucleic acid fragment of a cytokine-inducible promoter for Ig germline transcription, 5' of said CMV promoter.
- 14. The nucleic acid molecule of claim 13 wherein said cytokine-inducible promoter is an IL-4 inducible Is promoter.
- 15. The nucleic acid molecule of claim 12 selected from the group consisting of XF-1, XF-5a, XF-8, XF-2a, XF-2b, XF-6a and XF-6b.
 - 16. A switch vector comprising a nucleic acid molecule of claim 1.
 - 17. A switch vector comprising a nucleic acid molecule of 12.
- 18. A recombinant host cell stably transfected with the switch vector of claim 16.
- 19. A recombinant host cell stably transfected with the switch vector of claim 17.
 - 20. The host cell of claim 18 which is a mammalian cell.
 - 21. The host cell of claim 20, which is a Chinese Hamster Ovary (CHO) cell.
 - 22. The host cell of claim 20 which is a primary human B cell.
- 23. A method of monitoring immunoglobulin (Ig) class switch recombination (CSR), comprising



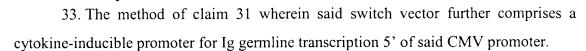
10

15

20

25

- (i) a first class switch region (S₁) nucleotide sequence of an upstream Ig locus under transcriptional control of a first promoter;
- (ii) a second class switch region (S₂) nucleotide sequence of an Ig locus downstream of said upstream Ig locus under transcriptional control of a second promoter, wherein said S₂ sequence serves as a region-specific substrate for CSR;
- (iii) a reporter gene nucleotide sequence encoding a reporter molecule interposed between said S_1 and S_2 sequences in reverse transcriptional orientation, and
- (iv) a promoter, downstream of said nucleotide sequence encoding said reporter molecule, allowing the expression of said reporter molecule only following switch recombination between said S_1 and S_2 sequences;
- (b) stably transfecting a mammalian cell with said switch vector; and
- (c) monitoring the expression of said reporter molecule in said mammalian cell, wherein such expression indicates CSR.
- 24. The method of claim 23 wherein said mammalian cell is a primary B cell or a B cell line.
- 25. The method of claim 24 wherein said B cell line is a human B lymphoma cell line.
- 26. The method of claim 25 wherein said cell line contains a single copy of said switch vector.
- 27. The method of claim 23 wherein said reporter molecule is Green Fluorescent Protein (GFP).
 - 28. The method of claim 27 wherein CSR is monitored by fluorescence microscopy.
 - 29. The method of claim 28 further comprising the step of quantifying CSR.
 - 30. The method of claim 29 wherein said CSR is quantified by flow cytometry.
 - 31. The method of claim 29 wherein said first promoter is a CMV promoter.
 - 32. The method of claim 29 wherein said second promoter is an SV promoter.



- 34. The method of claim 33 wherein said cytokine-inducible promoter is an IL-4 inducible Is promoter.
- 35. The method of claim 34 further comprising the step of culturing said cells in the presence of IL-4 and/or a stimulator of CD40 activity prior to monitoring CSR.

10

15

20

25

- 36. The method of claim 35 wherein said stimulator of CD40 activity is an anti-CD40 monoclonal antibody (mAb) or a CD40 ligand.
- 37. The method of claim 35 further comprising the step of exposing said cells to a candidate molecule, and determining the effect of said candidate molecule on GFP expression.
- 38. A method of monitoring immunoglobulin (Ig) class switch recombination (CSR) comprising
 - (a) providing a switch vector comprising, under transcriptional control of a promoter and in natural transcriptional orientation,
 - (i) a first class switch region (S₁) nucleotide sequence of an upstream Ig locus;
 - (iii) a second class switch region (S₂) nucleotide sequence of an Ig locus downstream of said upstream Ig locus; and
 - (iv) a reporter gene nucleotide sequence encoding a reporter molecule, interposed between said S_1 and S_2 sequences;
 - (b) incubating said switch vector with a cell-free nuclear extract from Igproducing cells or cells with Ig-producing potential; and
 - (c) detecting deletion of said reporter gene.
- 39. The method of claim 38 wherein said first class switch region sequence (S_1) is an $S\mu$ sequence and said second class switch region sequence (S_2) is an $S\epsilon$ sequence.
- 40. The method of claim 38 wherein deletion of said reporter gene is detected following transformation of said switch vector into a recombinant host cell.
- 41. The method of claim 40 wherein said recombinant host cell is a prokaryotic cell.

- 42. The method of claim 41 wherein said prokaryotic cell is an *E. coli* cell.
- 43. The method of claim 40 wherein said reporter gene is a lacZ gene.
- 44. The method of claim 43 wherein deletion of said reporter gene is detected by counting the white colonies obtained after transformation, in the presence of isopropyl-β-D-thiogalactoside (IPTG) and 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal).

- 45. The method of claim 38 wherein said Ig-producing cells are B lymphocytes.
- 46. The method of claim 45 wherein said B lymphocytes are of human origin.
 - 47. The method of claim 38 wherein said Ig-producing cells are primary B cells stimulated with CD40.
 - 48. The method of claim 38 wherein said S_1 and S_2 comprise G-rich, tandemly repetitive sequences.